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THE IMPACT OF *TARAXACUM OFFICINALE* AQUEOUS EXTRACTS ON WISH CANCER CELL LINE

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Objective: *to investigate the impact of aqueous extracts of Taraxacum officinale on the WISH (monolayer) cancer cell line. Chemical composition and pharmacological properties of dandelion are considered.* ***Methods:*** *this article considers microscopic method based on inhibition and percentage determination of cancer cell lines.* ***Results:*** *experiment shows that dandelion dried and fresh extracts (from root and leaves) can be used against cervix cancer cells – human WISH (monolayer) cell line.*

Key words: extraction; dandelion; *Taraxacum officinale*; WISH (monolayer) cell line; inhibition of cancer cell line.

Introduction. According to the World Health Organization, cancer is the second reason of death in the world; 9.6 million people over the world have died in 2018 year [1]. In Ukraine, according to the National Cancer Registry of Ukraine, every year 160-170 thousand people die. That is why, it is proposed to solve this problem with the help of phytotherapy by means of using extracts of medicinal plant raw materials.

Taraxacum officinale, also known as dandelion, is a herbaceous perennial plant of the *Asteraceae* family. Due to an extremely diverse number of biologically active compounds, its preparations in the form of aqueous extracts, infusions, tinctures, decoctions are used for the treatment and prevention of various health problems, including diseases of liver, gallbladder, gastrointestinal tract, kidneys, bladder and others [2, 3].

Plants of the genus *Taraxacum*, including *T. officinale*, are often used in traditional medicine for the cancer treatment. There are many studies that prove this possibility of dandelion [4–8].

Present study aims to evaluate whether aqueous extracts of dried and fresh vegetative organs (root and leaves) of the plant have an inhibitory effect on WISH cell line.

Materials and methods. The *Taraxacum officinale* root and leaves were collected in Chernihiv region during April 2019. They were washed under running tap water to remove traces and dust and then artificially dried at 40 °C.

Extraction of bioactive compounds from dried and fresh dandelion root and leaves was carried out using the following method [9]: 5 g of crushed plant material (degree of crushing 2-3 mm) with 100 mL of distilled water were added in a glass bottles with a capacity of 100 mL and kept in a water bath (water bath-thermostat with stirring WB-4MS) for 30 minutes at 55 °C. Then extracts were filtered through filter paper and a bacteriological filter «Epikriz» to prevent contamination by microorganisms.

We got six aqueous extracts: dried root and dried leaves extracts, mixture of dried extracts in a ratio 1:1, fresh root and fresh leaves extracts, mixture of fresh extracts in a ratio 1:1.

For the checking an anticancer activity of *Taraxacum officinale* we used WISH (monolayer) cancer cell line (Fig. 1). It was provided on LLC «PP «Biopharma» and obtained from the R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NASU. General characteristic of WISH (monolayer) cell line is represented in Table 1.

Table 1

General characteristics of WISH (monolayer) cell line

Organism	*Homo sapiens*, human
Tissue of origin	Cervix
Cell type	Epithelial cells
Growth properties	Monolayer
Description	Originally derived from human amnion tissue [10]. Has been used in virus studies; susceptible to vesicular stomatitis virus, adenovirus 3 and poliovirus. Useful in differentiating virulent and avirulent measles virus. This cell line was found to be indistinguishable from HeLa by STR PCR DNA profiling. Therefore, the cell line must be considered as derivative from HeLa. Ethnicity: black.
Nutrient medium	RPMI-1640 medium with the addition of 10% fetal bovine serum and 1% antibiotic-antimycotic solution (10000 MO/mL of penicillin, 10 mg/mL of streptomycin, 25 mg/mL of amphotericin B).

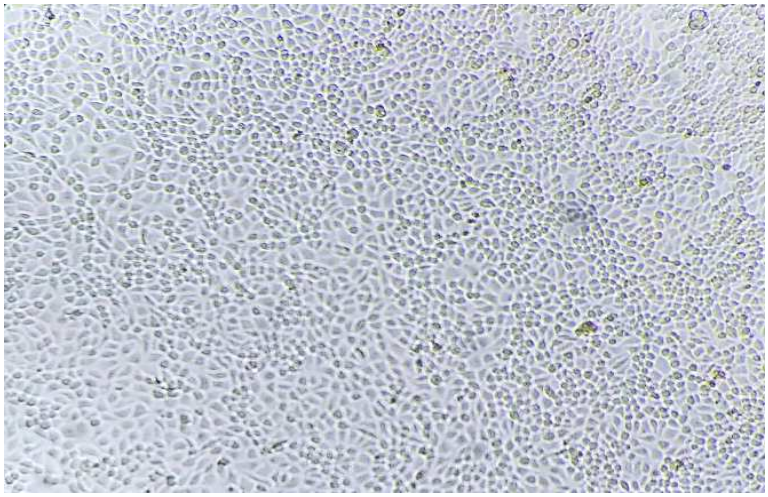
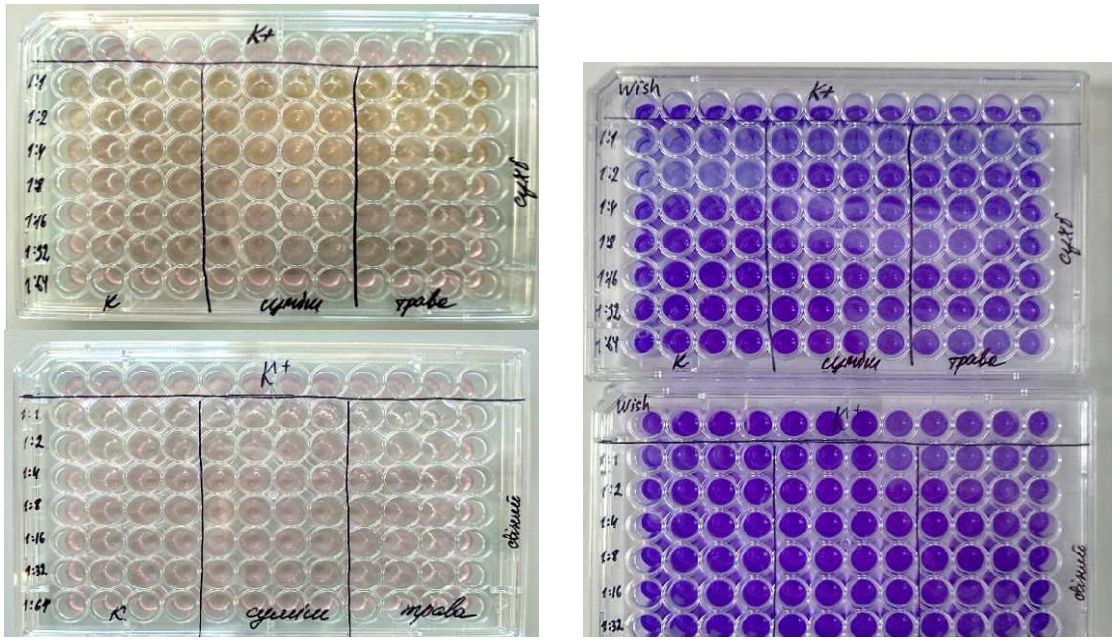


Fig. 1. WISH (monolayer) cell line under microscope, 100x

By mixing with a nutrient medium, the concentration of the cells in the suspension was adjusted to 3×10^5 cells/mL, poured by 100 µL into all wells of 96-well plates and incubated in the atmosphere of 5% CO_2 at 37 °C and humidity of 95-97 % (standard conditions for cell culture) for 6 hours for cells attachment.

WISH cell line was exposed to extracts of dandelion root and leaves in different concentrations for 48 hours (Fig. 2).



a **b**

Fig. 2. WISH cell line before (a) and after (b) the incubation

After incubation, 100 µL of the 0,1% crystal violet solution was added to each well to assess the cell growth. Dye solution was then dissolved in 100 µL of 96% alcohol solution, and the optical density of each well was measured at a wavelength of 540 nm. The spectrophotometrically determined values of optical density were converted to percentages (%) of cell viability by the following formula:

The cells not exposed to extracts served as a control (viability 100 %).

Statistical processing of the obtained data was carried out using MS Excel table processor.

Results. The results of investigation show that aqueous extracts of *Taraxacum officinale* have anticancer activity against WISH (monolayer) cell line (Table 2 and 3).

Table 2

Anticancer activity of dried dandelion extracts against WISH (monolayer) cell line

Dilution	Dried root extract	Mixture of extracts (1:1)	Dried leaves extract
K+	1,51	1,42	1,29
1:1	0,59	0,60	0,56
1:2	0,39	1,17	1,05
1:4	1,43	1,19	1,06
1:8	1,55	1,33	1,16
1:16	1,66	1,49	1,27
1:32	1,58	1,44	1,31
1:64	1,53	1,46	1,25

Table 3

Anticancer activity of fresh dandelion extracts against WISH (monolayer) cell line

Dilution	Fresh root extract	Mixture of extracts (1:1)	Fresh leaves extract
K+	1,92	2,04	2,11
1:1	1,07	1,19	1,24
1:2	1,60	1,79	1,97
1:4	1,69	1,95	2,07
1:8	1,65	1,84	2,03
1:16	1,66	1,86	1,98
1:32	1,65	1,87	2,00
1:64	1,60	1,88	1,91

Tables 2 and 3 represent the average values of optical density with wavelength 540 nm. K+ (control) is the average values of optical density of WISH cell line without any extract. B, C, D, E, F, G, H are the cells under corresponding dilutions 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 of dried and fresh dandelion extracts.

According to dynamic of changes in optical density after the influence of dried (Fig. 3) and fresh (Fig. 4) dandelion extracts, the best results of cancer cell inhibition are observed in the less diluted extracts (dilutions 1:1 and 1:2).

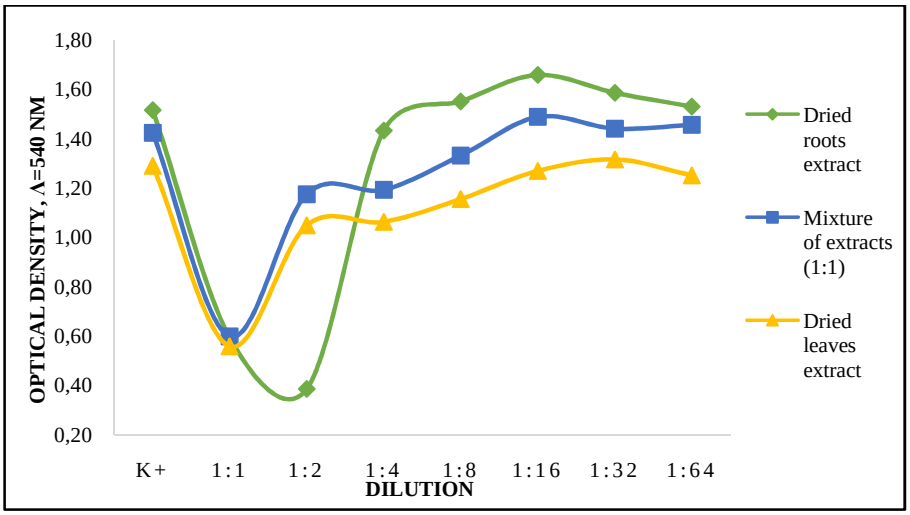


Fig. 3. Dynamic of changes in optical density after the impact of dried dandelion extracts

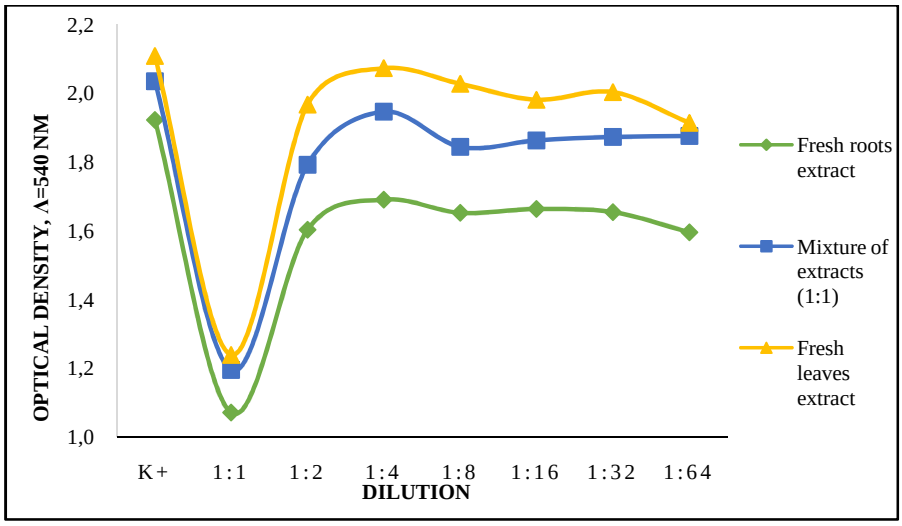


Fig. 4. Dynamic of changes in optical density after the impact of fresh dandelion extracts

As previously reported, the best results of inhibition of cancer cells are observed at dilutions of 1:1 and 1:2. So, Table 4 shows that at a dilution of 1:1 the percentage of alive cells after the impact of dried dandelion extracts (root, mixture and leaves) is 39,2 %, 42,0 % and 43,3 % respectively. At a dilution of 1:2 the best result is observed after the impact of dried dandelion root extracts and is 25,5 %.

Table 4

Percentage of cells viability after the impact of dried dandelion extracts, %

Dilution	Dried root extract	Mixture of extracts (1:1)	Dried leaves extract
1:1	39,2	42,0	43,3
1:2	25,5	82,5	81,3
1:4	94,5	83,7	82,4
1:8	102,3	93,5	89,6
1:16	109,4	104,5	98,4
1:32	104,6	101,2	102,0
1:64	100,9	102,2	97,0

Table 5 shows that at a dilution of 1:1 the percentage of alive cells after the impact of fresh dandelion extracts (root, mixture and leaves) is 55,7 %, 58,7 % and 58,7 % respectively.

Table 5

Percentage of cells viability after the impact of fresh dandelion extracts, %

Dilution	Fresh root extract	Mixture of extracts (1:1)	Fresh leaves extract
1:1	55,7	58,7	58,7
1:2	83,4	88,0	93,3
1:4	87,9	95,7	98,3
1:8	85,9	90,6	97,8
1:16	86,5	91,5	93,9
1:32	86,0	92,0	95,0
1:64	83,0	92,2	90,8

Also with the help of electronic microscope we may observe that WISH (monolayer) cell line is dead, because all cells are not in monolayer (Fig. 5) against the control (Fig. 1), where all cells are located on the surface.

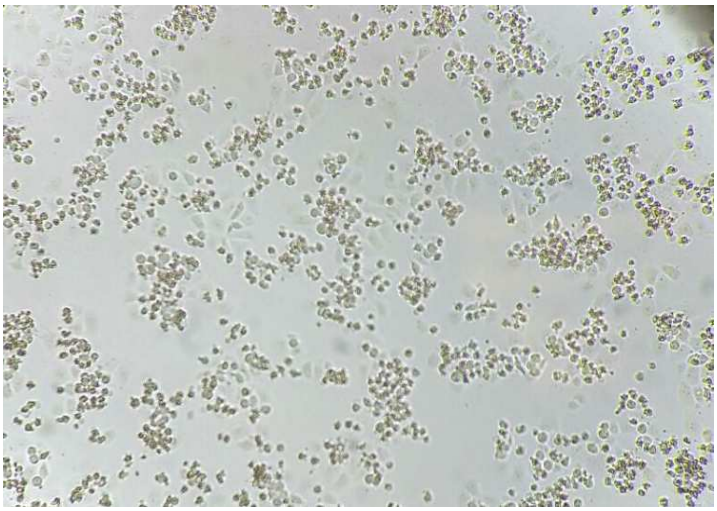


Fig. 5. Dead WISH (monolayer) cell line under dandelion extract, 100x

CONCLUSIONS

The article gives a general description of the chemical composition and pharmacological properties of *Taraxacum officinale*. The analysis of scientific research devoted to methods of determination of dandelion anticancer activity was carried out.

The impact of aqueous extracts from dried and fresh *Taraxacum officinale* (root and leaves) was investigated. The results of investigation show that the maximum inhibition of WISH cell line occurs under the impact of dried root extract at dilution of 1:2. The percentage of alive cells is 25,5 % in comparison with the control. The less inhibition occurs under the influence of dried dandelion extracts (root, mixture and leaves) at a dilution of 1:1. The percentage of alive cells is 39,2 %, 42,0 % and 43,3 % respectively.

The maximum inhibition of WISH cell line under the impact of fresh dandelion extracts (root, mixture and leaves) occurs at a dilution of 1:1. The percentage of alive cells is 55,7 %, 58,7 % and 58,7 % respectively. At other dilutions, inhibition of the cell line occurs in less degree.

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ВПЛИВ ВОДНИХ ЕКСТРАКТІВ *TARAXACUM OFFICINALE* НА РАКОВУ КЛІТИННУ ЛІНІЮ WISH

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Мета: *дослідити вплив водних екстрактів кульбаби лікарської (Taraxacum officinale) на ракову клітинну лінію WISH (моношар). Розглянуто хімічний склад та фармакологічні властивості кульбаби.* ***Методи:*** *у статті розглядається мікроскопічний метод, заснований на інгібуванні та відсотковому визначенні ракових клітинних ліній.* ***Результати:*** *експеримент показує, що водні екстракти висушеної та свіжої кульбаби лікарської (з коренів і листя) можуть бути використані проти ракових клітин шийки матки – людської клітинної лінії WISH (моношар).*

Ключові слова: *екстракція; кульбаба; Taraxacum officinale; клітинна лінія WISH (моношар); інгібування ракової клітинної лінії.*

ВЛИЯНИЕ ВОДНЫХ ЭКСТРАКТОВ *TARAXACUM OFFICINALE* НА РАКОВУЮ КЛЕТОЧНУЮ ЛИНИЮ WISH

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Цель: *исследовать влияние водных экстрактов одуванчика лекарственного (Taraxacum officinale) в раковую клеточную линию WISH (монослой). Рассмотрены химический состав и фармакологические свойства одуванчика.* ***Методы:*** *в статье рассматривается микроскопический метод, основанный на подавлении и процентном определении раковых клеточных линий.* ***Результаты:*** *эксперимент показывает, что водные экстракты высушенного и свежего одуванчика лекарственного (из корней и листьев)*

могут быть использованы против раковых клеток шейки матки – человеческой клеточной линии WISH (монослой).

Ключевые слова: *экстракция; одуванчик; Taraxacum officinale; клеточная линия WISH (монослой); ингибирование раковой клеточной линии.*